Remarks/Argument

Claims 1, 2, 5, 7-9, 11, 14 and 17-22 are pending in the application.

The amendment to the specification corrects the spelling of an author's name on a citation. Since the citation information and the paper's authors are factual and readily known to at the time of filing, this amendment does not introduce new matter.

After entry of this Response, claims 1, 2, 5, 7-9, 11, 14 and 17-22 will be pending in the application. Based on the above changes and the following remarks, Applicant respectfully requests reconsideration of the claims and withdrawal of the pending rejections.

Response to Section 112, 1st paragraph rejections

Claims 1, 2, 5, 7-9, 11, 14 and 17-22 are rejected under 35 U.S.C. §112, 1st paragraph as allegedly failing to comply with the written description requirement. It is alleged that no adequate written description is provided of an RNA that is homologous to any target gene, or any portion of any target gene, that will disrupt the expression of any target gene because the specification does not provide the specific structure of an RNA that would correspond with the function of being homologous to any target gene.

Preliminarily, it is well-settled law that the written description requirement is viewed in light of the state of the art and skill of the practitioner at the time the application was filed. In *Vas-Cath, Inc. v. Mahurkar,* 19 USPQ2d 1111 (Fed. Cir. 1991), the Court of Appeals for the Federal Circuit traced the development of the written description requirement under 35 U.S.C. §112, first paragraph. The *Vas-Cath* Court, in a unanimous opinion, noted approvingly that in a written description analysis, "[t]he primary concern is factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure." *Vas-Cath*, 19 USPQ2d at 116 (quoting *In re Wertheim*, 191 USPQ 90, 96 (C.C.P.A. 1976)). After discussing the policy reasons underlying the requirement, the Court set forth the standard for the written description requirement:

> The purpose of the "written description" requirement is broader than to merely explain how to "make and use"; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. . . . The test for sufficiency of support in a parent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter."

Vas-Cath, 19 USPQ2d at 1117 (emphasis added) (quoting *Ralston Purina Co. v. Far-Mar-Co., Inc.*, 227 USPQ 177, 179 (Fed. Cir. 1985)). *Accord University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997). Therefore, it is well-settled that the knowledge of those skilled in the art informs the written description inquiry.

In determining the sufficiency of support in a disclosure with respect to the written description requirement, "it is not necessary that the application describe the claimed invention in *ipsis verbis*; all that is required is that it reasonably convey to persons skilled in the art that, as of the filing date thereof, the inventor had possession of the subject matter later claimed by him." *In re Edwards*, 196 USPQ 465, 467 (C.C.P.A. 1978) (citing *In re Lukach*, 169 USPQ 795 (C.C.P.A. 1971); *In re Driscoll*, 195 USPQ 434 (C.C.P.A. 1977)). More recently, the Court of Appeals for the Federal Circuit, in *In re Kaslow*, 217 USPQ 1089, 1096 (Fed. Cir. 1983), citing *In re Edwards*, emphasized:

> The test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter, rather than the presence or absence of literal support in the specification for the claim language.

More recently, in *In re Alton*, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996), the court of Appeals for the Federal Circuit pointed out that literal support is not required in order to satisfy the written description requirement:

> If a person ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate written description requirement is met. For example, in *Ralston Purina Co. v. Far-Mor-Co., Inc.*, 227 USPQ 177, 180 (Fed. Cir. 1985), the trial court admitted expert testimony about known industry standards regarding temperature and pressure in "the art of both farinaceous and proteinaceous vegetable materials." The effect of the testimony was to expand the breadth of the actual written description since it was apparent that the inventor possessed such knowledge of industry standards of temperature and pressure at the time the original application was filed.

More recently, in *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997) cert. denied, 523 U.S. 1089 (1998), the Court of Appeals for the Federal Circuit stated:

> "In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus."

The court reasoned that factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406; MPEP 2163 II(A)(3)(a)(i).

When species of a genus are claimed, only a representative number of species must be adequately described. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. Furthermore, the description of a representative number of species does not require the description to be of such specificity that it need provide individual support for each species of a genus. *In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994).

The Examiner has the burden of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. MPEP 2163. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed. *In re Wertheim*, 541 F.2d 257, 262, 191 USPQ 90, 96.

Therefore, it is clear that the invention need not be described in *ipsis verbis, i.e.*, literally, for purposes of the written description requirement under 35 U.S.C. §112, first paragraph. Rather, what is needed is that the skilled artisan understand, based upon the disclosure in the specification as filed and the knowledge imputed to the skilled artisan at the time the specification was filed, that the inventor had possession of the claimed subject matter.

Applicant respectfully submits that one skilled in the art, upon reading the specification as filed, would have understood what was meant by an RNA that is homologous to any target gene and thus that the Applicant was in possession of the claimed invention.

The Examiner asserts that the specification lacks specific guidance concerning what level of nucleotide sequence homology is required of an RNA for use in the instant invention and what the structure is of an RNA that would correspond with the function of targeting any gene.

The degree of homology for the claimed invention is inherently disclosed in the examples in the specification and this would be recognized by one skilled in the art. Specifically, the examples in the specification teach making RNA strands for annealing

to make the dsRNA molecule using in vitro transcription reactions. The *in vitro* transcription reactions use a DNA template which provides the sequence to which a homologous RNA strand is desired and RNA polymerases, specifically SP6 RNA polymerase and T7 RNA polymerase. These are well-known polymerases which have known error rates. The error rate for SP6 RNA polymerase is approximately 10^{-4} mutation per nucleotide and for T7 RNA polymerase is 10^{-4} to 10^{-5} mutation per nucleotide. See for instance Boyer et al. (1992) *Proc Natl Acad Sci* 89:6919-6923; Agapov et al. (1998 *Proc Natl Acad Sci* 95:12989-12994; and Pugachev et al. (2004) *J. Virol.* 78:1032-1038. Given these known error rates and the number of nucleotides in the transcripts made, one skilled in the art can calculate the number of errors expected in a population of RNA molecules. For the 828 nucleotide strands transcribed in the Example, the strand transcribed by SP6 RNA polymerase is expected to have roughly .08 mutation and the strand transcribed by T7 RNA polymerase is expected to have roughly .008 mutation (using 10^{-5} error rate). Presuming that each RNA strand will have independent errors (that is errors will not be at the same positions), one can add together the two numbers of errors expected to get the number of errors expected in a dsRNA. In a given 828 base pair strand then, one would expect 0.088 mutations. Obviously a mutation cannot be fractional; what these calculations indicate however is that in a population of, for instance, 1000 molecules of the 828 base pair dsRNA, one expects to find about 88 mutations. In other words, 912 of the 1000 molecules are expected to be 100% homologous while 88 are slightly less than 100% homologous. Thus, the specification inherently teaches that the homology for the dsRNA used in the inventive method approaches 100%.

Furthermore, the specification provides teachings on the length of the dsRNA homologous to a target gene. In the examples, a dsRNA molecule of 828 bp (see p.12, lines 28-31) homologous to cKitR was employed in the method to induce RNAi. This number therefore provides an upper limit on what sized dsRNA is successfully used in the method claimed. A lower limit on the size of the dsRNA is evident in the logic used

in designing antisense molecules. Given the size of the human genome (haploid genome about 3 x 10^9 bases), any sequence that is 17 nucleotides or longer has a high probability of being unique. See p. 47, middle column, under "Theoretical limits of specificity" in Branch (1998). Since the mechanism of RNA interference relies on homology to a particular sequence, one skilled in the art recognizes that the minimum dsRNA size to achieve target specificity is 17 nucleotides. Therefore, the range of size for dsRNA appropriate for use in the instant invention is 17 to about 830 basepairs.

The specification discloses four general methods of obtaining dsRNA for use in the instant invention. See p.1, line 30- p. 2, line 28. A preferred method is set forth in the example. See p. 12, line 28-p. 13, line 12. Specifically, the two strands of RNA are made in an *in vitro* transcription reaction using a DNA template comprising a fragment of the target gene subcloned into an expression vector having an SP6 promoter and a T7 promoter. *In vitro* transcription reactions using the cognate RNA polymerases followed by strand annealing and purification yields the dsRNA molecule homologous to the target gene.

There are many human genes whose overexpression is related to a disease state and whose sequence was known at the time of filing (*e.g.*, HER-2/neu). Importantly, however, the method of producing the dsRNA disclosed in the specification does *not* require the skilled practioner to actually know the detailed sequence of the target gene. At the time the application was filed, there were a huge number of genes that have been cloned but are not sequenced or not. It is routine molecular biology for one skilled in the art to sub-clone a gene or gene fragment into an appropriate expression vector without knowing its sequence. (See for instance chapters 8 and 9 in "Molecular Cloning, A Laboratory Manual, Second Edition" by Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY, 1989). These expression vectors can be amplified and used in *in vitro* transcription reactions in order to produce the RNA strands that are annealed into dsRNA homologous to a target gene for use in the inventive methods.

In summary, the specification teaches the degree of homology for the dsRNA used in the claimed invention, as well as the range of sizes for the dsRNA (from 17 to about 830 bp) and the method of making dsRNA homologous to the target gene. Based upon the disclosure in the specification as filed and the knowledge imputed to the skilled artisan at the time, a skilled artisan would indeed understand that the applicant had possession of the claimed subject matter.

Applicant respectfully requests reconsideration and withdrawal of the 35 U.S.C. § 112, first paragraph, written description rejection.

Claims 1, 5, 7, 9, 11, 14 and 17-22 are rejected under 35 U.S.C. 112, 1st paragraph as lacking enablement for using RNAi to disrupt gene expression *in vivo*.

The Examiner states that the specification is enabled only for using RNAi *in vitro* to practice methods of disrupting KitR gene expression in human cancer cell lines using Kit dsRNA (KdsRNA). It is noted that the Examiner acknowledges repeatedly that Applicant has demonstrated that RNAi can be induced in human cells *in vitro* throughout the Office Action.

The Examiner maintains that the state of the prior art at the time of filing with reference to Agrawal et al. 2000, Branch 1998, Green et al. 2000 and Jen et al. 2000, indicates that one of skill in the art of RNAi would still require specific guidance to practice the claimed methods *in vivo* as claimed, to overcome the major impediment how to achieve specific delivery of particular nucleic acids to effect a particular therapeutic outcome. Applicant respectfully disagrees.

As a first matter, the Examiner states on page 9, first full paragraph, that Applicant's argument that conclusions drawn from studies on frog cells have little bearing on human cells amounts to Applicant conceding that RNAi results even *in vitro* are unpredictable and cannot be applied one species to another. Applicant respectfully points out that this broad generalization is *solely* the Examiner's. Applicant did not argue this broad generalization at all. Indeed, the Examiner's attention is drawn to page 4, lines

17-23 in the specification in which Applicant states there are links between the genetic analysis of RNAi from diverse organisms and the biochemical model of RNAi that is emerging from Drosophila *in vitro* systems.

The Examiner maintains that the working examples in the present specification, which show inhibition of KitR expression by RNAi in two human cancer cell lines, are insufficient to enable the full scope of the claimed methods. However, there is no requirement that the working examples, of themselves, fully support a claimed invention. Rather, the specification *taken as a whole* must be enabling. In re Barr, 170 USPQ 330 (CCPA 1971).

Here, the working examples show that RNAi can be induced in CHP 100 neuroepithelioma (melanoma) and HL-60 leukemia cells, which represent two different human cancer cell lines from different tissues and developmental origins. Because the instant application shows that RNAi can be induced in such widely divergent human cancer cell lines, one skilled in the art would reasonably believe that RNAi could be induced in any human cell. Thus, the present specification (including the working examples) contains ample direction on how to practice the full breadth of the claimed RNAi therapeutic method.

To be enabling, a specification must teach one skilled in the art how to make and use the claimed invention without undue experimentation. As reviewed at length in the response dated July 13, 2004, the specification clearly and extensively teaches how to make dsRNA (or pharmaceutical compositions of the dsRNA) for administration to a human subject and numerous ways to deliver the dsRNA. Indeed, two of the articles cited by the Examiner also discuss the various successful ways nucleic acid is delivered *in vivo* to cells.

Jen et al. (2000), *Stem Cells* 18: 307-319 details numerous techniques for delivering nucleic acids to target cells *in vivo*, including delivery of naked nucleic acid and delivery with agents such as cationic liposomes, cationic porphyrins, polyethylenimine, fusogenic peptides and artificial virosomes. Jen et al. note on p. 97

that "[m]any of the limitations to antisense studies outlined earlier have been relatively easily dealt with," and that "the *in vivo* delivery of (antisense oligonucleotides) appears to be more efficient that that which occurs *in vitro*."

Greene et al. (2000) provide numerous examples of *in vivo* studies in which antisense oligonucleotides were successfully delivered to human patients. See, *e.g.*, pgs. 99-102 of Greene et al., which detail the successful delivery of antisense oligonucleotides to target cells in humans by: intravenous infusion (to treat lymphoma, steroid-dependent Crohn's disease and HIV); subcutaneous administration (to treat non-Hodgkin's lymphoma and prostate cancer); and intravitreal injection (to treat AIDS-related cytomegalovirus infection).

Furthermore, recent work has shown that dsRNA can be successfully delivered *in vivo* using delivery methods as taught in the instant specification. On page 10, lines 19-24, the specification teaches delivery means including intravenous. Song et al. (2003) delivered dsRNA homologous to *Fas* by hydrodynamic intravenous injection into mice. *Fas* encodes the Fas receptor in hepatocytes (liver cells) and renders the hepatocytes vulnerable to Fas apoptosis which is the basis for hepatic injury from a variety of causes including autoimmune hepatitis. The intravenous injection resulted in specific reduction of Fas mRNA in the hepatocytes. See p. 347, second column, first full praragraph and Figure 1, panels b and c. Furthermore, the dsRNA injection protected the mice from fulminant hepatitis and hepatic fibrosis induced by concanacalin A (Con A). See pp. 349-350 and Figures 2 and 3. Thus, a therapeutic effect is achieved as a result of systemic intravenous injection of dsRNA homologous to a target gene.

A second example of systemic administration of dsRNA in mammalian cells has been shown. Soutschek et al. (2004) *Nature* 432:173-178 injected various dsRNA homologous to apolipoprotein B (apo B) into tails of mice. While the cholesterol-modified dsRNA did prove more serum-stable, it is important to note that the non-cholesterol modified dsRNA resulted in a detectable reduction in apoB mRNA. See page 174, Figure 2, panels b-d.

While both of these demonstrations are in mice and the claims are directed to human cells, mice have long been primary models for human therapeutics. For instance, the induction of hepatitis by Con A in mice as used in Song et al. (2003) is an art-recognized model for human hepatitis. See, for instance, Kaneko et al. (2000) *J. Exp. Med.* 191:105-114. Indeed, mice are described by NIH as "the preeminent surrogate model system for human disease and normative biology." See: National Institutes of Health, National Center for Research Resources, Comparative Medicine Resources Directory, Mutant Mouse Regional Resource Centers [online], [retrieved 2005-01-23]. Retrieved from the Internet URL:www.ncrr.nih.gov/ncrrprog/cmpdir/RODENT.asp. As such, *in vivo* work in mice serves to enable *in vivo* work in humans.

These post-filing *in vivo* examples of dsRNA effectively reducing gene expression support that Applicant's specification is enabling for *in vivo* methods in human cells. It is clear that undue experimentation is not necessary to carry out Applicant's invention.

Thus, the present specification gives ample guidance on how to make the claimed dsRNA, and how to use the dsRNA to effect RNAi of a target gene in a human cell. The data presented in the present specification show that RNAi-specific effects can be produced without unduly triggering the PKR response. Subsequent work *in vivo* in mammalian cells indicate that the claimed invention will achieve a therapeutic effect *in vivo*. The unique nature of RNAi, including the ability of dsRNA to form multiple siRNA "guide sequences" and to induce the destruction of hundreds or thousands of target mRNA molecules, avoids the problems encountered with antisense technology. Moreover, one skilled in the art can follow the teachings of the specification and practice the claimed RNAi methods without undue experimentation. Claims 1, 5, 7, 9, 11, 14 and 17-22 are thus enabled for treating an RNA-based disorder or disease in a human subject, by administering dsRNA to induce gene-specific RNAi. Applicant, therefore, respectfully requests that the rejection of claims 1, 5, 7, 9, 11, 14 and 17-22 under 35 U.S.C. §112, 1st paragraph be withdrawn.

Response to Section 102(e) rejections

Claims 1, 2, 5, 7-9, 11 and 22 are rejected under 35 U.S.C. §102(e) as being anticipated by Fire et al. (USP 6,506,559).

The claimed invention is drawn to a method of disrupting target gene expression in a human cells by exposing the cell to a dsRNA homologous to the target gene thereby initiating RNA interference.

Fire teaches the use of dsRNA in methods in cells of inhibiting expression of a target gene *in vitro.* While the Fire specification provides a virtual laundry list of possible cells for use with the method, the reduction to practice is limited to the invertebrate animal, *C. elegans.* The disclosure regarding dsRNA induced inhibition in higher order, vertebrate cells was sheer speculation. Indeed, Fire authored at least two papers well after the effective filing date of '559 in which the very speculative nature of RNAi in mammalian cells, such as mice and humans, was made clear. In Montgomery and Fire (1998) TIG 14:255-258, the authors end the article with a discussion of the expected response in mammalian cells to exposure to dsRNA. Specifically, they note that a mammalian cell exposed to dsRNA "unleashes a vehement but somewhat non-specific response leading to general translational arrest" related to PKR protein kinase (p. 258 sentence bridging columns 1 and 2). The authors thus conclude that "any gene-specific interference by dsRNA in PKR-proficient mammalian cells would be dependent on a transient lapse in the PKR response, or a controlled level of dsRNA that was incapable of activating PKR." The authors further indicate that while there are numerous suggestions in the literature of RNA-mediated interference in gene expression control, it is unknown whether the RNA that induces the interference is single-stranded or double-stranded. See p. 258, first full paragraph, second column. These statements clearly indicate that one skilled in the art did not expect dsRNA to induce RNA interference in human cells.

The speculative nature of RNAi in human cells at the time of filing of '559 and before the inventive work in the instant application is further emphasized in Fire (1999) *Trends Genet.* 15:358-363. On pp. 362-363 under "Real-world applications: what about us?", Fire states that "one could certainly hope that RNA-triggered silencing would exist in vertebrates." He then states that "although this hope is not ruled out by any current data, the simple protocols used for invertebrates and plant systems are unlikely to be effective." These statements clearly indicate that at the time of filing of the '559 patent, one skilled in the art, the inventor himself, did not believe his claimed methods would work in mammalian cells.

Contrary to the wisdom and expectations of the skilled practioner, Applicant set about to induce RNA interference in human cells by exposing human cells to dsRNA homologous to a target gene. Contrary to the wisdom and expectations of the skilled practioner, Applicant succeeded. Applicant's seminal discovery was neither anticipated nor obvious in light of the art. Applicant therefore requests the withdrawal of this rejection.

Conclusion

Based on the foregoing, all pending claims are believed in condition for allowance. An early and favorable action toward that end is earnestly solicited.

Respectfully submitted,

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